Metabolic Imaging of the Neurovascular Interface in Cerebrovascular Disease using Positron Emission Tomography

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Abstract:

Cerebrovascular disease encompasses a range of pathologies affecting different components of the cerebral vasculature and brain parenchyma. Large artery atherosclerosis, acute cerebral ischaemia, and intracerebral small vessel disease all demonstrate metabolic processes that are key to pathogenesis. Although structural imaging has been a mainstay of stroke clinical care and research, it has limited ability to detect these pathophysiological processes *in vivo*. Positron emission tomography (PET) provides a means to detect and quantify metabolic processes in each facet of cerebrovascular disease non-invasively. The use of PET has helped shape the understanding of key concepts in cerebrovascular medicine, including the vulnerable atherosclerotic plaque, salvageable ischaemic penumbra, neuroinflammation and selective neuronal loss after ischaemic insult, and the relationships between chronic hypoxia, neuroinflammation, and amyloid deposition in cerebral small vessel disease. This review considers how the ability to image these processes at the neurovascular interface has contributed to our understanding of cerebrovascular disease and facilitated translational research to advance clinical care.

Key Points:

- PET allows non-invasive detection and quantification of inflammation, microcalcification, and hypoxia that are associated with plaque rupture in carotid atherosclerosis.
- The temporospatial evolution of acute ischaemia and secondary neuroinflammation can be visualised using PET.
- PET has elucidated the complex interaction between cerebral small vessel disease, neuroinflammation, and amyloid deposition, demonstrating different patterns within vascular cognitive impairment.
- Metabolic imaging with PET provides not only improved understanding of disease mechanisms but can also provide sensitive endpoints in clinical trials and has the potential to improve clinical risk-stratification.

Introduction:

Positron emission tomography (PET) is increasingly used in neuroimaging research strategies, both to image the cerebral arteries and the brain parenchyma, and has contributed significantly to our understanding of cerebrovascular disease and translational research to advance clinical care. Although structural imaging has been a mainstay in stroke research and clinical care, such imaging provides only limited insight into important physiological processes. Improved understanding of the role of intra-plaque inflammation in triggering carotid atheroma rupture and thromboembolism means that a purely anatomical approach based solely on the degree of luminal stenosis is over-simplistic and risks overlooking the role of symptomatic, but non-stenosing, atheroma[1,2]. Assessment of plaque physiology *in vivo* may facilitate the development of better treatment approaches, risk-stratification of plaque to guide clinical management, and improved monitoring of treatment effects from therapy in clinical care and research trials[3,4]. In acute stroke, metabolic imaging has shaped the concept of the salvageable penumbra, providing the basis for radiological assessments of perfusion to guide acute reperfusion therapy, as well as potentially extending the time window for such therapy[5]. Finally, PET imaging has improved our understanding of pathophysiology in small vessel disease – particularly the interaction between chronic cerebrovascular disease, amyloid deposition, and cognitive impairment – that may guide the development of new treatments for what is effectively an untreatable disease currently.

This review considers different facets of cerebrovascular disease – including the metabolic pathophysiology of carotid atherosclerosis, metabolic changes in the penumbra in the acute period, and the neuroinflammatory changes associated with small vessel disease and vascular cognitive impairment – and how PET has elucidated these pathologies at the neurovascular interface to facilitate clinical exploitation.

Principles of PET:

PET allows detection of physiological processes by using targeted radioligands. Radioligands target physiological processes through a number of mechanisms, including cellular uptake (e.g. the glucose analogue ^{18}F-fluorodeoxyglucose, FDG[6]),

incorporation into products of metabolism (e.g. incorporation of ^{18}F-sodium fluoride, NaF, into microcalcification[7]), or via targeting cell surface receptors (e.g. binding to peripheral benzodiazepine receptors by *N*-butan-2-yl-1-(2-chlorophenyl)-*N*-methylisoquinoline-3-carboxamide, PK11195[8]) (TABLE 1). Once positron-emitting radioligands accumulate in a region of interest (ROI), positrons rapidly encounter electrons within local tissues, resulting in annihilation reactions. The release of gamma photons is subsequently detected and quantified by scintillation detectors and co-localised to anatomical regions through co-registration with computed tomography (PET/CT) or magnetic resonance imaging (PET/MRI).

PET in Vascular Risk:

Atherosclerosis and the Vulnerable Plaque:

Carotid atherosclerosis is estimated to underlie 20% of ischaemic strokes. Atherosclerotic plaque rupture with subsequent thromboembolism is a key event triggering ischaemia, and may occur independently of the degree of luminal stenosis[9]. The atherosclerotic plaque, formed by the infiltration of circulating monocytes with subsequent maturation to macrophages and development of a necrotic core, is contained within a fibrotic cap[1]. Metabolic processes including inflammation, microcalcification (calcium deposition smaller than 50 micrometers detected by *ex vivo* μCT[10]), and hypoxia, represent a mix of chemical and mechanical triggers that increase the risk of fibrous cap rupture, the so-called "vulnerable plaque."[1,11-13]

Conventional clinical imaging is limited in its ability to detect these pathophysiological processes and subsequently most clinical imaging remains structural, using the degree of luminal stenosis to determine management. Magnetic resonance imaging (MRI) has facilitated improved detection of morphological features associated with plaque vulnerability (necrotic core size, fibrous cap thickness, intraplaque haemorrhage)[14], though such imaging remains technically demanding and has limited ability to detect microcalcification. In contrast, PET provides a highly sensitive non-invasive imaging modality to detect pathophysiological processes predisposing to plaque rupture.

Quantification of tracer uptake is measured using standardised uptake values (SUV), where measured tracer uptake in a ROI is adjusted according to injected dose and patient weight. Although used widely in solid organs, this method has shortcomings when used in vascular imaging as it fails to consider the effect of tracer pooling within the blood[15]. Tissue-to-background ratios (TBR) corrects for this by dividing SUV within the vessel wall by the SUV in a mid-luminal venous region[16]. The most accurate method remains the subject of debate[17].

Different PET tracers have been used to image different processes in atherogenesis: macrophage-mediated inflammation (FDG and DOTATATE), microcalcification (NaF), and hypoxia (^{18}F-fluoromisonidazole, FMISO).

Plaque inflammation:

Fluorodeoxyglucose:

FDG was the first radiotracer employed in atherosclerosis imaging after incidental uptake in carotid arteries was noted in individuals undergoing cancer staging PET[18]. Early clinical studies demonstrated the capacity of FDG-PET to identify culprit carotid atheroma *in vivo*[19] (FIG. 1), with subsequent histological studies showing FDG uptake correlated strongly with CD68+ macrophage burden in plaques[16]. Higher FDG uptake in symptomatic carotid plaques is also associated with recurrent ischaemic stroke, independent of the degree of luminal stenosis[20,21]. A potential mechanism for this is ongoing microembolisation, which was observed (using transcranial Doppler ultrasound) in plaques with higher FDG uptake, though the study was limited to a small number of symptomatic individuals and lacked correlation to clinical outcomes[22].

Large histological studies have demonstrated high-risk morphological features associated with plaque rupture[23]. FDG uptake is associated with a number of these features, and may provide a means of assessment *in vivo*[24,25]. Detection of vulnerable plaque has also informed our understanding of atherosclerosis previously considered subclinical, with examples of non-stenotic but morphologically high-risk atheroma demonstrating increased FDG uptake[2,26]. Introduction of PET/MRI is likely to provide

further detail about the interaction between metabolic and morphological processes through simultaneous image acquisition.

FDG-PET supports the hypothesis of atherosclerosis as a systemic disease. Inflammation within one artery is associated with increased inflammation within neighbouring arterial regions[27]. Higher carotid uptake is also observed in the presence of with dental inflammation[28], raised low-density lipoprotein and total cholesterol levels[29], diabetes mellitus[30], and metabolic syndrome[31]. Carotid FDG uptake in individuals with recent acute coronary syndrome is higher than in chronic stable angina, supporting a systemic upregulation of plaque inflammation that may contribute to the clinical phenomenon where acute coronary events and ischaemic stroke occur within close temporal proximity[32]. Furthermore, a recent FDG-PET study supports a relationship between the brain and systemic atherosclerosis, with stress-related amygdala activity found to be associated with increased bone marrow activity, vascular inflammation on FDG-PET, and cardiovascular events[33].

FDG-PET studies have indicated important interactions involved in plaque progression. Increased inflammation in aortic atheroma is independently associated with increased risk of developing macrocalcification[34], and the presence of macrocalcification within carotid arteries appears to propagate inflammation[35].

As well as identifying plaque vulnerability, FDG-PET has furthered understanding of therapeutic mechanisms[36]. An observed reduction in FDG uptake in an asymptomatic cohort, independent of the change in low-density lipoprotein, supports the pleiotropic effects of statins[37]. This reduction occurs within weeks of starting a statin, with apparent dose-responsive effects[38].

FDG-PET is not without its weaknesses. The high sensitivity may be compromised by a reduced specificity when uptake in neighbouring metabolically active tissues (e.g. lymph nodes, vocal cords) spills-over into adjacent vascular ROIs, resulting in artefacts. As FDG is taken up in competition with circulating glucose, it is necessary to fast prior to PET/CT, and high circulating glucose will interfere with FDG uptake. Finally, renal impairment may lead to prolonged circulating radiotracer that will result in artificially low TBR readings[39]. Adoption of common FDG-PET methodological

standards and development of more specific radiotracers for inflammation will likely minimise these effects and reduce inter-study variability[15,40].

Alternative and emerging radiotracers:

^{11}C-*N*-methyl-*N*-[1-methylpropyl]-1-[2-chlorophenyl]-isoquinoline-3-carboxamide (PK11195) targets translocator protein 18-kDa (TSPO), formerly known as the peripheral benzodiazepine receptor (PBR), which is expressed on macrophages. PK11195 uptake co-localises with macrophages on histology[41,42], and is raised in symptomatic carotid atheroma[43]. However, animal studies found no significant difference in PK11195 uptake between atheroma and healthy vessel wall[44]. This is likely due to ubiquitous expression of TSPO, and consequently PK11195's use for atheroma imaging has been limited. However, caution must be exercised when applying tracer pharmacokinetics in animal models to humans, due to potentially different receptor expression. Second-generation TSPO radioligands have been developed with greater specificity and favourable signal-to-noise ratios, though their accuracy has been limited by variable receptor binding in humans that is discussed further below.

A promising new radiotracer is ^{68}Ga-DOTATATE ([1,4,7,10-tetraazacyclododecane-N,N′,N″,N‴-tetraacetic acid]-d-Phe1,Tyr3-octreotate) (FIG. 2). Somatostatin receptor subtype-2 (SST_2) is upregulated on the cell surface of macrophages, to which DOTATATE demonstrates high specific binding activity. DOTATATE uptake differentiated between symptomatic and asymptomatic carotid plaques in pilot work, and correlated strongly with histological macrophage burden and Framingham risk scores[45]. DOTATATE's superior specificity relative to FDG results in improved signal-to-noise ratio, particularly in the coronary arteries. Tracer production does not require a cyclotron meaning imaging is much cheaper than using FDG and does not require pre-scan fasting.

Microcalcification:

In addition to inflammation triggering plaque rupture via metalloproteinase-mediated mechanisms, mechanical destabilisation also plays a role. Microcalcification is

mediated by osteoblast-like cells derived from vascular smooth muscle cells, a process driven by inflammatory cytokines while microcalcification may in turn provoke further inflammation[46]. Microcalcification within the fibrous cap increases plaque stress and predisposes to rupture[47,48].

CT and MRI are unable to detect microcalcification as it falls below its spatial resolution. In contrast, ^{18}F-NaF accumulates at sites of microcalcification, where ^{18}fluorine is exchanged for the hydroxyl group in hydroxyapatite[49]. Histological validation in carotid endarterectomy samples indicated fluoride co-localised to pathological mineralisation[7].

Incidental NaF arterial uptake on cancer staging PET was detected in regions with and without visible macrocalcification. This, alongside the absence of NaF uptake in some areas of macrocalcification, supports NaF binding to sites of active mineralisation rather than simply reflecting accumulation in macrocalcification[50].

Increased NaF uptake is seen in recently ruptured atheroma and unruptured plaques with high-risk features *in vivo*, indicating tracer uptake reflects increased microcalcification and not simply increased binding area following plaque rupture[51]. Similar results have been seen in individuals with stable angina, with a history of cardiovascular events, and higher Framingham risk scores[52]. Recent studies of carotid atheroma in transient ischaemic attacks and minor strokes have found increased NaF uptake in culprit atheroma[53,54]. Interestingly, Vesey et al. reported that although NaF uptake was higher in culprit versus asymptomatic atheroma, FDG did not differ significantly. Similar findings were reported in a smaller dual tracer study of symptomatic carotid atheroma[55]. This contrast to the earlier FDG-PET/CT studies supports the concept that the different tracers are targeting two distinct but related processes.

NaF uptake correlates with a range of vascular risk factors; age, hypertension, hypercholesterolaemia, smoking, diabetes, and a history of prior cardiovascular events[56]. It is also associated with histological characteristics of a vulnerable plaque, namely cell death, presence of necrotic core, and macrophage infiltration[51].

A dual tracer study in asymptomatic individuals undergoing oncology staging demonstrated only 6.5% of arterial lesions showing concomitant FDG and NaF uptake; supporting inflammation and microcalcification as two related but distinct processes in atherosclerosis[57]. The presence of macrocalcification appears to alter this relationship between inflammation (FDG) and microcalcification (NaF). In a cohort of individuals with myeloma, FDG and NaF uptake did not correlate in non-calcified lesions, while lower but concordant uptake was noted in mildly calcified lesions (r=0.7) and severely calcified lesions (r=0.4)[58]. These PET studies suggest a temporal evolution of atherogenesis with distinct phases and interplay between inflammation, microcalcification, and macrocalcification.

Hypoxia:

^{18}F-fluoromisonidazole (FMISO) is a 2-nitroimidazole that undergoes selective bioreduction and accumulates within hypoxic cells[59]. Hypoxia occurs due to increasing oxygen demand from foam cells exacerbated by increasing necrotic core size, plaque thickness, and distance from the luminal wall[60]. Higher FMISO uptake has been observed in symptomatic than asymptomatic atheroma and correlated with FDG uptake, consistent with hypoxia as either a contributing factor to inflammation or playing a direct role in FDG uptake[61].

Implications:

The ability to define absolute uptake thresholds for stability is currently limited by the typically small size of studies and heterogeneous methodologies. van der Valk et al. propose a FDG maximum TBR of 1.84 as the upper limit of physiological uptake in the carotid, corresponding to the 90th centile of controls[62]. However, it is important to note that in this study the average BMI was 25, which may increase tracer uptake as described above. Marnane et al. found a one unit increase in symptomatic artery FDG mean SUV resulted in an adjusted (for age and degree of stenosis) hazard ratio of recurrent stroke at 90 days of 6.1 (95% CI 1.3-28.8, p=0.02), with a mean SUV threshold of 1.85 providing an optimal balance of sensitivity (61.5%) and specificity (78.7%) to discriminate between individuals at low and high risk of recurrence[20]. For DOTATATE, a maximum TBR of greater than 2.66 had 87.5% sensitivity and 78.4%

specificity to detect culprit coronary segments[45]. For PET to be utilised clinically for risk stratification, risk thresholds need to be established for tracer uptake and future work should look towards harmonising study protocols to facilitate meta-analysis.

Atheroma PET imaging is highly reproducible with high inter-reporter agreement across radiotracers[52,63]. The high sensitivity means subtle differences in tracer uptake are readily detectable and require fewer participants to studies to reach sufficient power. Rather than using conventional clinical endpoints, such as recurrent events, PET can be used to measure metabolic endpoints in drug trials. The dal-PLAQUE phase 2b study used FDG-PET endpoints to test the effects of dalcetrapib, a cholesteryl ester transfer protein that raises high-density lipoprotein. Although the primary outcome was negative, the study demonstrated the use of metabolic endpoints in atherosclerosis trials is feasible[4,64]. Such an approach may be adopted to understand the underpinning mechanisms and establish proof of principle responses to intervention, prior to larger studies assessing clinical response.

PET in Acute Ischaemic Stroke:

PET has also provided valuable mechanistic insights in acute infarction, establishing the concept of the "salvageable penumbra." During acute cerebral ischaemia, the penumbra represents a region of neuronal dysfunction that may be reversed if cerebral perfusion is restored (in contrast to the infarct core where neuronal dysfunction is irreversible)[65,66]. This phenomenon provides the target for acute reperfusion therapy in acute ischaemic stroke, as well as underpinning the use of CT-perfusion and MRI to identify penumbra to guide this therapy.

Ischaemic injury to the brain provokes a reactive gliosis involving activation of astrocytes and microglia, the resident macrophages of the central nervous system. Although neuroinflammation is generally seen as contributing to pathophysiology in most neurodegenerative conditions, it is believed to play a complex dual role following ischaemia. The post-infarct microglial response involves two arms: pro-inflammatory M1 and anti-inflammatory M2 microglial subtypes, with an early polarisation from M2 to M1 subtypes in peri-infarct regions[67]. M1 microglia exert neurotoxic effects through production of reactive oxygen species and pro-

inflammatory proteins, which may themselves perform a dual role. Matrix metalloproteinase-9 (MMP-9) exacerbates ischaemic damage by promoting blood-brain barrier disruption and neuronal death in the early stages post-infarct, but may promote brain regeneration and neurovascular remodeling in latter phases[68,69]. The M2 microglial subtype is predominantly protective, with associated neuronal survival[70]. Imaging using PET has helped elucidate the mechanisms underlying the neuroinflammatory response to ischaemia and how this response may affect the brain beyond the site of infarct.

Penumbra:

^{15}O-PET:

Early ^{15}O-PET measuring tissue perfusion (cerebral blood flow, CBF) and cerebral oxygen consumption (CMRO2) identified three distinct groups within a cohort of ischaemic stroke patients: extensive areas of greatly reduced CBF and CMRO2, extensive moderate CBF reduction but only focal areas of marked CMRO2 reduction, and increased CBF with only focal areas showing reduced CMRO2 (FIG. 3). Although small studies, these patterns showed proof of principle and matched clinical outcomes, with poor, variable, and good outcomes respectively, independent of initial neurological status[71,72]. Acute ^{15}O-PET has found salvageable penumbra of 10-52% of the infarct volume up to 17 hours post-infarct[73]. The presence of penumbra beyond conventional clinical thrombolysis windows has led some to call for prospective studies assessing the selection for thrombolysis on a case-by-case physiological basis (most likely assessed by CT-perfusion; a CT-based dynamic sequential scanning technique of the movement of contrast through the vasculature that can be used to visualise CBF and cerebral blood volume, the mismatch of which represents the penumbra as described above) rather than absolute time cut-offs[74,75].

Co-registered ^{15}O-PET and diffusion-weighted imaging (DWI), an MRI sequence imaging the diffusion of water molecules with impaired movement resulting in generation of increased contrast, implies the DWI lesion includes not only an infarct core but also penumbra[76]. This was supported by further work mapping CBF, CMRO2, and oxygen ejection fraction (OEF) onto DWI lesions, which showed a

heterogeneous pattern of oxygen metabolism within the DWI lesion, as well as variation between individuals, and supported the patterns of oxygen metabolism seen in the earlier ^{15}O-PET work[71,77], indicating that some of DWI lesion may be reversible and represent salvageable tissue. Furthermore, "misery perfusion" – regions exhibiting low CBF, high OEF, but only moderately reduced CMRO2 – was the predominant pattern in the DWI lesion and was also seen outside of the DWI ROI, suggesting that there is regional variation determining different perfusion thresholds[77,78]. The use of voxel-based mapping in ^{15}O-PET has allowed thresholds for irreversible tissue damage to be estimated: 8.43 ml/100 ml/min for CBF and 0.87 ml/100 ml/min for CMRO2. Infarcts with higher proportions of voxels below these thresholds were associated with larger final infarct volumes and poorer neurological outcomes at two months[79]. The reversibility of DWI lesions, and the salvageable tissue it may contain, has important implications for both research methodology (which may use DWI volume as endpoints) and clinical practice.

Fluoromisonidazole:

Penumbral mapping has advanced with the introduction of newer PET radiotracers. FMISO, accumulating within hypoxic but viable cells, was seen in the peripheries of the infarct in 9 of 13 of individuals scanned within 48-hours of infarction but none of those scanned at 6-11 days[80]. Studies using MRI data to strictly define the infarct core found FMISO uptake in both the penumbra and core[81]. FMISO studies have demonstrated temporospatial evolution of infarction, with tracer uptake moving from the centre to the periphery with increasing time post-infarct[82,83]. This suggests that there is a region of the core that, while unsalvageable and destined to infarct, still demonstrates sufficient activity for FMISO uptake. Conversely, FMISO uptake is also seen in tissue beyond the symptomatic tissue on co-registered MRI, suggesting oligaemic tissue also experiences hypoxia[81,84].

FMISO uptake in the acute stage may also be able to predict outcomes, with 80% of those with uptake showing an increase in infarct volume compared to no infarct growth in those without FMISO uptake[85]. This is unsurprising given the nature of the tissue represented by FMISO uptake, but given the limited ability of MRI to differentiate between penumbra and benign oligaemia, this method for quantifying

vulnerable tissue may have important implications for prognostication. A higher proportion of surviving tissue measured by FMISO-PET at 12 hours and between 12-48 hours post-stroke was associated with better neurological recovery at 3 months[86]. Supporting the findings of ^{15}O-PET, FMISO uptake has also been seen up to 131 hours after the stroke[85]. These findings suggest potentially salvageable tissue beyond current clinical intervention windows.

Flumazenil:

Impaired functional recovery despite successful reperfusion of penumbra is an important clinical issue. ^{11}C-flumazenil (FMZ) offers insights into this phenomenon by allowing *in vivo* imaging of selective neuronal loss (SNL) following ischaemic stroke. SNL differs from "pannecrosis" – the widespread necrosis of cells including glial cells, neurons, and white matter – in that there is selective death of neurons but other cells and the extracellular matrix are preserved[87]. This selective loss of neurons is associated with poorer functional recovery as these activated neurons, found predominantly in the peri-infarct cortical areas, determine neuroplasticity after stroke[88].

FMZ is an antagonist to the benzodiazepine binding site of gamma aminobutyric acid-type A (GABA-A) receptors, mainly found on neurons. Consequently, SNL results in decreased FMZ uptake. FMZ-PET shows potential in differentiating unsalvageable tissue from viable penumbra, with the region of decreased FMZ uptake corresponding with final infarct volume[89]. The ability to differentiate between viable and non-viable tissue has implications for reperfusion therapy. In 11 patients undergoing thrombolysis with decreased CBF, only the 4 individuals with decreased FMZ uptake showed evolution to an infarct[90].

FMZ-PET has suggested significant SNL in salvaged penumbra, with the reduction in FMZ binding proportional to acute-stage hypoperfusion and resulting in reduced neuronal activation on MRI[91,92]. Such findings have clear ramifications for the functional recovery in such salvaged penumbra, as well as a potential mechanism to explain mismatch of functional deficit and radiological findings in the sub-acute to chronic setting.

Similar findings associated with carotid stenosis have been seen with decreased FMZ binding borderzone territories with reduced CBF but without evidence of infarction, suggesting that haemodynamic compromise from large artery disease may be associated with SNL[93].

FMZ-PET has informed our understanding of oedema formation after middle cerebral artery infarction. The use of infarct volume as the conventional predictor for malignant oedema has a low positive predictive value. In contrast, FMZ-PET within 24-hours of infarct found that volume of severe ischaemia, rather than total perfusion deficit, predicted development of malignant oedema with a high positive predictive value[94].

Neuroinflammation:

PK11195:

Imaging neuroinflammation *in vivo* is possible through the use of PK11195 targeting TSPO expressed on microglia (and upregulated upon their activation) but not astrocytes following ischaemic damage[95]. The temporal pattern of microglial activity in humans post-infarct with PK11195-PET shows minimal binding within 72-hours, increasing within a week before falling by 25-30 days[96]. The spatial distribution of tracer uptake mirrored that described in hypoxia, with uptake initially seen in the core at 7-14 days before the peri-infarct zone showed a higher degree of uptake in the sub-acute phase. Importantly, there was also increased tracer uptake in the contralateral hemisphere at latter time points, suggesting that neuroinflammation extends beyond the infarct[96,97] (FIG. 4). Similar temporal patterns were seen by Gerhard et al., though spatial evolution of PK11195 uptake showed movement from the peri-ischaemic region to increasing overlap with the MRI-defined infarct region[98]. This study, along with others, suggests longterm microglial activation remote from the infarct up to 150 days post-infarct[98,99].

It is important to note that differences in spatial patterns may be due to a combination of small samples and slightly different time intervals, potentially catching different

stages of systemic infiltration. More recent animal studies demonstrated a lack of PK11195 in the infarct core, instead localising to normoperfused peri-infarct regions and co-localising with increased FDG uptake suggesting increased energy demand in these regions[100]. However, caution should again be exercised when comparing binding behavior between species.

Remote microglial activation likely indicates Wallerian degeneration along anatomically-connected neural networks[101,102]. Co-registering PK11195 with diffusion tensor imaging (DTI), which images the direction of water diffusion in order to identify neural tracts, after subcortical infarction showed uptake occurred only in an antegrade direction and only if the pyramidal tracts were affected, suggesting the spread of activated microglia is specific to affected tracts rather than a non-specific disseminated response[103,104]. The extent of remote microglial activation appears independent of infarct size or clinical severity. One clinical study has shown differing clinical patterns depending upon the location of activated microglial activity; initial PK11195 uptake in the tracts was associated with improved clinical outcomes while persistent tracer uptake in the infarct was associated with poorer clinical outcomes[104].

Second generation TSPO radioligands:

Quantification of these temporospatial patterns of PK11195 uptake is challenging due to non-specific binding, resulting in sub-optimal signal-to-noise ratios, and difficulty in defining a reference region for comparison, hence study results should be interpreted with this caveat. Newer second-generation TSPO radioligands have increased specificity (resulting in improved signal-to-noise) and advantageous pharmacokinetics. However, an emergent physiological factor has limited their wider adoption. ^{11}C-PBR28 was shown to have improved specificity by a dual PK11195 and PBR28 rat study[105], but subsequent work comparing receptor binding between animal models and humans found receptor binding to be markedly lower in humans compared to monkeys, and that there was variation in the human binding with some showing no specific binding[106]. Subsequent studies have revealed polymorphism in the gene Ala147Th that results in high-affinity and low-affinity binders, with heterozygous individuals displaying intermediate affinity binding[107,108]. In contrast, PK11195 does not appear to have variable affinity as it binds to a different site on the

TSPO[8]. These findings show it is important to exercise caution when comparing tracer pharmacokinetics between different animal species and humans, as well as human subject comparisons.

The short half-life of ^{11}C-labeled radiotracers has important implications for research and clinical use. PK11195's half-life of 20.38 minutes means that an on-site cyclotron is essential[109]. In contrast, the 110-minute half-life of ^{18}F-labeled radiotracers makes imaging possible without a cyclotron onsite. One example is ^{18}F-*N,N*-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-α]pyrimidin-3-yl)acetamide (DPA-714). DPA-714 rapidly accumulates within the infarct, with detectable uptake reaching a peak 5 minutes after tracer injection, and supports earlier findings that the extent of microglial activation does not correlate with the size of the infarct[110]. No direct comparison between PK11195 and DPA-714 has been performed in humans, though direct comparison in rat models shows DPA-714 to have a superior signal-to-noise ratio[111,112]. ^{11}C-vinpocetine demonstrated higher uptake in infarct core, peri-infarct region, and contralateral hemisphere that PK11195 in human subjects post-infarct, though the difference did not reach statistical significance[113,114]. Newer TSPO ligands, such as ^{18}F-GE-180, have shown superiority to PK11195 in animal models but their application within humans is yet to be seen. Furthermore, it remains to be seen if DPA-714, vinpocetine, and GE-180 are also affected by variable TSPO affinity.

Implications:

Although PK11195 and second-generation TSPO ligands provide means of detecting activated microglia, they are unable to differentiate between M1 and M2 subtypes. Understanding the pattern *in vivo* of these different subtypes, and how they relate to the imaging results discussed above, would provide important mechanistic information to further understand their different roles in post-stroke neuroinflammation.

The use of PET/MRI will likely enable greater accuracy through simultaneous acquisition of DTI and tracer co-localising compared to previous studies where these scans were performed on different machines at different time points.

PET in Chronic Small Vessel Disease:

Chronic cerebral small vessel disease (SVD) – disease in the perforating cerebral arteries and arterioles – is a major cause of vascular cognitive impairment (VCI). VCI may occur as a result of several different vascular pathologies characteristic of SVD: microinfarcts from arteriolar occlusion, lacunar infarction, or diffuse white matter injury through incomplete infarction[115]. Furthermore, SVD may interact with neuroinflammation, microhaemorrhages, and amyloid deposition in the development of cognitive impairment. Although structural imaging can confirm the presence of white matter hyperintensities (WMHs), not all WMHs will be symptomatic. PET has helped the understanding of their natural history, and provided potential imaging surrogate markers of disease in research and clinical applications.

Formation and Implications of WMH:

Cognitive decline in VCI is attributed to damage of the white matter, particularly subcortical. The degree of cognitive impairment has only a limited association with the degree of WMHs seen on MRI, but has a stronger correlation with hypoperfusion and hypometabolism[116]. This hypometabolism can be visualised with FDG-PET, where focal reductions in signal tend to be asymmetric, which can be differentiated from the global reduction seen in Alzheimer's dementia[117].

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) provides an effective model of SVD. In these individuals, the regional cerebral metabolic rate of glucose measured by FDG-PET is significantly reduced in cortical and particularly subcortical structures compared to controls[118]. Both a reduction in CBF and neuronal loss may contribute to these findings. ^{15}O-PET-measured CBF reduces in a dose-dependent manner for individuals heterozygous and homozygous for a CADASIL-causing R133C mutation compared to controls[119].

Individuals with essential hypertension and WMHs who experienced cognitive decline over the three-year follow-up had significantly lower CBF on ^{15}O-PET at

baseline compared to those without cognitive decline, likely a consequence of small vessel remodeling[120]. This reduction in CBF and intermittent hypoxia is in turn likely to trigger a neuroinflammatory response.

Chronic Neuronal Pathology and Neuroinflammation:

The activation of microglia, particularly their role in MMP-mediated blood-brain barrier disruption, is important in chronic cerebrovascular disease. The microglial response following infarction is described above, but microglial activation also accompanies chronic SVD in human post-mortem studies[121].

Polarisation of microglia from protective M2 to neurotoxic M1 subtypes represents a potential therapeutic opportunity in SVD. Minocycline, a tetracycline antibiotic with anti-inflammatory action, selectively inhibits the polarisation of pro-inflammatory M1 microglia[122]. In animal models of focal infarction, minocycline reduced microglial activation, improved neurogenesis, reduced infarct volumes, and improved functional outcomes[123,124]. In animal models minocycline significantly reduced white matter damage, most likely through inhibition of hypoxia-inducible factor-1α and MMP-9 and consequent reduction in blood-brain barrier disruption[125,126]. PK11195 studies are currently underway assessing whether similar results are seen in humans.

Links between SVD and Alzheimer pathology:

PET has furthered understanding of interactions between SVD and Alzheimer's pathology observed in epidemiological studies and post-mortem studies[127]. Accumulation of amyloid precursor protein (APP) has been detected in areas of chronic hypoperfusion and acute infarction[128], and amyloid beta (Aβ) protein localised to areas of SVD in human post-mortem specimens[129]. Overexpression of APP is associated with a greater reduction in cerebral blood flow and larger volumes of infarcts in transgenic mice[130]. This mechanism has proven harder to demonstrate in humans, though PET using N-methyl-[^{11}C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole (PiB) has suggested processes not observable in structural imaging. PiB is an analogue of thioflavin-T that binds with high affinity to aggregated amyloid[131]. Individuals with recent infarcts were found to have elevated PiB uptake in

the peri-infarct region in the sub-acute phase[132] (FIG. 5). However, this study, like earlier animal studies, is limited in its ability to conclude whether Aβ contributed to infarct development or whether it is a consequence of ischaemia.

FDG uptake is lower in WMHs in individuals with abnormally high cerebrospinal fluid Aβ protein concentrations, implying an association between amyloid dysmetabolism and WMHs[133]. Longitudinal studies using PiB and MRI suggest that amyloid burden can interact with SVD burden in individuals with VCI, particularly in semantic word fluency and global dementia rating[134]. The burden of WMH is associated with increased progression of amyloid burden assessed by PiB, suggesting SVD results in impaired amyloid clearance[135].

PiB uptake has been found to be associated with a four-fold increase in the risk of developing incident dementia within six months after stroke/TIA[136]. PET may also be able to discriminate between pathology in early- and late-onset subcortical VCI, where early-onset disease was associated with a higher number of lacunes but late-onset disease showed higher PiB uptake, despite no significant difference in WMH volumes[137]. As well as suggesting different pathological bases, this study also found differences in structural changes, with a decrease in mean cortical thickness and hippocampal volume associated with PiB uptake. Amyloid burden measured by PiB appears to affect cortical atrophy and cognitive impairment without mediation by the white matter network, in contrast to SVD that did result in network disruption[138]. Similarly, dual PiB and MRI studies have shown periventricular WMH volume, but not PiB uptake, was associated with gait disturbance in SVD[139].

The above shows potential utility of PiB-PET to delineate pure models of subcortical vascular dementia from Alzheimer pathology in mixed dementia. In a large PET study, two thirds of those diagnosed clinically with vascular dementia did not show PiB uptake, and this cohort performed better on memory testing but worse on frontal function tests than individuals with PiB-positive Alzheimer's disease[140].

Cerebral amyloid angiopathy:

PiB also has a potential role in the assessment of cerebral amyloid angiopathy (CAA), a chronic cerebral vascular disease characterised by infiltration of Aβ protein with associated microhaemorrhages, macrohaemorrhages, and microinfarcts, predominantly in a lobar distribution. PiB uptake appears increased in individuals with probable CAA in this cortical pattern[141]. CAA was observed to contribute to the development of WMH in SVD, likely through angiopathy-induced hypoxia, with global PiB uptake and WMH being strongly correlated in individuals with CAA. However, there was no similar relationship for PiB uptake in Alzheimer's disease, suggesting that the finding in CAA relates directly to the angiopathy[142].

Limitations:

Serial longitudinal imaging using PET/CT is limited by radiation exposure: a 250 MBq FDG-PET/CT of the carotids involves an additional radiation exposure of 5.5 mSv. Consequently, its use clinically in primary prevention for risk-stratifying atheroma identified through screening is limited by the uncertainty of when to scan.

As discussed above, although FDG-PET/CT is highly sensitive for inflammation, it has a lower specificity owing to tracer uptake in other metabolically active tissue. This is compounded by the limited spatial resolution of PET (3mm). Both of these considerations result in the potential for a partial volume effect, where there is difficulty differentiating tracer activity within an ROI from that of neighbouring tissues due to "spill-in" or "spill-out" (where signal from the tissue of interest falls outside of the ROI). This may be reduced by designing protocols that minimise physiological uptake in surrounding structures (keeping patients silent and nil by mouth, ensuring blood sugar control) and reduce movement artefact (appropriate head/neck supports). Partial volume error may also be reduced through the use of geometric transfer matrices and algorithms, where tracer uptake is matched to a voxel-based analysis from a higher resolution imaging modality helps restrict signal quantification to a specific region[143,144].

The availability, cost, and radiation exposure involved in PET studies involving human subjects has typically resulted in studies with small sample sizes. The sensitivity of PET means that small physiological changes may be detected,

facilitating statistically significant differences to be detected using these small numbers. However, it can be difficult to draw conclusions about clinically-relevant thresholds (e.g. level of plaque rupture) due to the small samples and potential selection bias of retrospective studies that limit the ability to perform robust multi-variable analysis. Differences in methodology, tracer administration, reconstruction of images, and tracer uptake measures further limit direct comparisons, with SUV values found to vary by a factor greater than three in some carotid FDG-PET/CT studies[17]. Currently, the body of cerebrovascular PET studies should be viewed as indicating important physiological mechanisms and general trends in disease patterns, with the potential for clinical application if the above considerations can be overcome.

Conclusions and future applications:

Although widespread clinical use of PET remains unlikely for the reasons described above, there may be potential clinical applications for assessing treatment responses in particularly high-risk arteriopathic individuals. For widespread clinical adoption, the use of PET for determining treatment in a randomised control trial would be required. However, the strength of PET is its ability to elucidate disease mechanisms, identify novel therapeutic targets, and its use as a potentially powerful tool for providing outcome measures in therapeutic trials. The dal-PLAQUE study has already shown how PET can be used effectively in atheroma drug trials. Similar approaches are currently being used in evaluating neuroinflammatory responses to treatment. Beyond pharmacological monitoring there is potential to elucidate the mechanisms underlying stroke rehabilitation. For example, post-infarct rehabilitation appears to reduce the proliferation of microglia around the infarct with improved functional recovery in animal models[145].

Newer PET ligands continue to be developed with superior specificity or for targeting new processes of interest. Epoxide hydrolase (sEH), an enzyme inactivating epoxyeicosatrienoic acids that have vasoactive and anti-inflammatory properties, is implicated in neuronal damage and has been found to be elevated in individuals with VCI[146]. *N*-(3,3-diphenylpropyl)-6-^{18}F-fluoronicotinamide (FNDP) targets sEH with a specificity of 80-90% in animal models[147]. PET tracers to the α7 nicotinic acetylcholine receptor (expressed on neurons, astrocytes, microglia, and endothelial

cells) have shown proof-of-principle applications for use in both intracerebral and vascular imaging[148,149]. Radiotracers specific for apoptosis, such as those targeting caspase-3 within the apoptotic pathway, are also in development[150].

It is important that the field consider adopting uniform methodologies to allow direct comparison of results and to facilitate meta-analysis with larger samples. Despite the first vascular FDG-PET studies having been performed 15 years ago, calls for harmonisation of methods persist[17,19]. As the number of radiotracers increases for imaging the different facets of cerebrovascular disease, it is vital that similar standardisation occur but at a much earlier stage.

PET is a versatile *in vivo* imaging technique that can provide important mechanistic insights across the range of neurovascular pathologies in cerebrovascular disease. The improved structural imaging offered by MRI in PET/MRI is likely to provide improved anatomical co-registration and simultaneous acquisition of both structural/morphological and metabolic imaging. This has the potential to advance our understanding of both vascular and intracerebral pathophysiology in cerebrovascular disease.

Competing interests:

Nil.

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Tracer	Molecular target	Cellular/physiological target	Technical considerations
^{18}F-FDG	Cellular uptake	Inflammation/metabolism	High sensitivity but potential for sub-optimal signal-to-noise ratios
^{68}Ga-DOTATATE	Somatostatin receptor subtype-2 (SST_2)	Macrophages	High specific binding activity
^{18}F-NaF	Hydroxyapatite	Microcalcification	Potential spill-over from vertebrae or mandible
^{18}F-FMISO	Selective reduction in hypoxia	Hypoxia	Long tracer uptake time (120 – 180 minutes)
^{11}C-PK11195	TSPO	Macrophages/microglia	Short half-life, sub-optimal signal-to-noise intracerebrally
^{11}C-PBR28	TSPO	Macrophages/microglia	Variable affinity binders and short half-life
^{18}F-DPA-714	TSPO	Macrophages/microglia	Superior signal to noise ratio but paucity of human data
^{11}C-vinpocetine	TSPO	Macrophages/microglia	Superior signal to noise ratio but paucity of human data and short haf-life

^{18}F-GE-180	TSPO	Macrophages/microglia	Superior signal to noise ratio but paucity of human data
^{11}C-FMZ	GABA-A receptor	Neurons	Short half-life
^{11}C-PiB	Analogue of thioflavin T	Amyloid	Short half-life

Table 1: Radiotracers used in vascular and cerebrovascular PET studies.